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Polypeptide Chain Initiation and Stepwise Elongation with *Artemia* Ribosomes and Factors*

(*Artemia salina*/embryos/cysts/brine-shrimp eggs/poly(U))

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ABSTRACT **The supernatant initiation factor from *Artemia salina* embryos promotes, besides the AUG-dependent binding of fMet-tRNA$_f$, the poly(U)-dependent binding of *N*-acetylPhe-tRNA to 40S ribosomal subunits; the bound *N*-acylaminoacyl-tRNA reacts directly with puromycin upon addition of 60S subunits. Both the binding reaction and the synthesis of *N*-acylaminoacyl-puromycin occur in the absence of GTP or other ribonucleoside triphosphates. To a smaller extent, the factor also mediates the 40S ribosomal binding of Met-tRNA$_f$ and Phe-tRNA; in this case, the bound aminoacyl-tRNA is less reactive with puromycin. After the poly(U)- and supernatant factor-dependent binding of *N*-acetylPhe-tRNA to 40S subunits at low Mg^{2+} concentration, binding of a second aminoacyl-tRNA (Phe-tRNA), with ensuing formation of the first peptide bond, is dependent upon the addition of the 60S subunit, elongation factor EF-1, and GTP. Further growth of the polypeptide chain requires translocation and is, therefore, dependent upon the addition of elongation factor EF-2. As with the *Escherichia coli* system, once requirements for translation of the third codon have been met, no further additions are necessary for elongation of a peptide chain.**

In previous papers (1, 2), we reported on an initiation factor from postribosomal supernatants of embryos of the brine shrimp, *Artemia salina*, and its relation to factors from rat-liver supernatant (3–6) and rabbit-reticulocyte ribosomal washes (7) described by other investigators. The *Artemia* factor and the *Escherichia coli* initiation factor IF-2 are not interchangeable (2) but, as previously noted (1), the reaction promoted by *Artemia* factor, namely the AUG-dependent binding of fMet-tRNA$_f$ to eukaryotic 40S ribosomal subunits, shares certain properties with the analogous reaction catalyzed by IF-2 in bacterial systems: (*a*) it occurs on the small ribosomal subunit, (*b*) it is sensitive to edeine and aurintricarboxylic acid, and (*c*) the bound fMet-tRNA$_f$ is converted directly to fMet-puromycin upon addition of the large subunit. However, the eukaryotic reaction is GTP-independent.

In this paper, we report that, in further analogy to IF-2 (8), the *Artemia* factor also promotes the poly(U)-dependent binding of *N*-acetylPhe-tRNA to 40S subunits, and the bound *N*-acylaminoacyl-tRNA is directly converted to *N*-acetylPhe-puromycin after addition of 60S subunits. Furthermore, the *Artemia* factor catalyzes, at low Mg^{2+} concentrations, not only the reported (1, 3, 5, 6) binding of Phe-tRNA, but also that of Met-tRNA$_f$ to 40S subunits. However, the unacylated derivatives are bound to a lesser extent than the *N*-acylated ones, and the bound aminoacyl-tRNA is less reactive with puromycin. The poly(U)-directed synthesis of *N*-acetylPhe-(Phe)$_n$ by the *Artemia* system requires (*a*) supernatant factor for formation of the 40S initiation complex, (*b*) the 60S subunit, elongation factor EF-1, and GTP, for binding of the second aminoacyl-tRNA and ensuing synthesis of the first peptide bond, and (*c*) elongation factor EF-2 for further growth of the chain.

MATERIALS AND METHODS

Ribosomal Subunits and Factors. A. salina ribosomal subunits (1) and the *A. salina* supernatant initiation factor (2) were prepared as outlined in preceding papers. The isolation and assay of partially purified chain-elongation factors EF-1 and EF-2 from *A. salina* was described (1). They were free of supernatant initiation factor. In order to remove traces of EF-2 contaminating the EF-1 fraction used in this work, use was made of the sensitivity of eukaryotic EF-2 and the insensitivity of EF-1 to SH-binding reagents (9). Contaminating EF-2 was rendered inactive by prior incubation of EF-1 fractions with 20 mM *N*-ethylmaleimide for 30 min at 25°. The unreacted *N*-ethylmaleimide was inactivated with excess (40 mM) dithiothreitol.

Assays. The assay for AUG-dependent binding of fMet-tRNA$_f$ or Met-tRNA$_f$ to *Artemia* 40S subunits was as described (2)†, with either *E. coli* f[^{14}C]Met-tRNA (330 cpm/pmol), 20 pmol; or *E. coli* [^{14}C]Met-tRNA$_f$ (400 cpm/pmol), 16 pmol. Incubation, 30 min at 0°. This assay is conducted at 0° because as noted (1), little or no 40S ribosomal binding of fMet-tRNA$_f$ is observed at 25°. Our assumption (1) that this is due to low stability of the AUG–40S–fMet-tRNA$_f$ complex is borne out by the fact that, when the temperature is shifted up from 0 to 25°, there is rapid loss of ribosome-bound radioactivity unless the more stable 80S complex is formed by addition of the 60S subunit (Table 1).

For assay of poly(U)-dependent *N*-acetylPhe-tRNA or Phe-tRNA binding, the samples contained, in a volume of 60 μl, 90 mM Tris·HCl buffer (pH 7.5) (25°), 120 mM KCl, 7.0 mM magnesium acetate, 2 mM dithiothreitol, 0.3 A_{260} unit of *Artemia* 40S ribosomal subunits, 1 A_{260} unit of poly(U), and either *E. coli N*[^{14}C]acetylPhe-tRNA, 12 pmol; or [^{14}C]Phe-tRNA, 11 pmol (each 670 cpm/pmol). Incubation was for 30

* This is Paper no. III in the series "Polypeptide Chain Initiation in Eukaryotes," Paper no. II is ref. 2.

† Through an oversight, the addition of AUG, 0.05 A_{260} unit, to the components of the assay was missing in this paper.

TABLE 1. *Stability of the fMet-tRNA–AUG–80S ribosome complex at 25°*

Min of incubation	Ribosomal binding of f[^{14}C]Met-tRNA (pmol)	
	No 60S subunits added	60S subunits added
0	2.9	2.9
1	3.0	2.8
10	0.1	1.9
30	0.03	1.9

Eight equal samples were incubated, under the conditions of the standard assay for 40S ribosomal binding of f[^{14}C]Met-tRNA, for 30 min at 0°. Then 0.65 A_{260} unit of 60S subunits was added to each of four samples, while the other four received an equal volume of buffer. One set (one sample with, one without 60S subunits) was assayed immediately for fMet-tRNA binding, while each of the remaining sets was incubated at 25° before assay. Net binding values are given after subtraction of a blank, amounting to about 20% of the net values, obtained in the absence of supernatant factor.

min at 25°. Unless otherwise stated, the *Artemia* supernatant factor preparation used was that described in the preceding paper (2); 0.26 μg/sample was used. The ribosome-bound radioactivity was determined as described (1). The determination of *N*-acylaminoacyl-puromycin or aminoacyl-puromycin synthesis and of protein was also described (1).

Formation of the *N*-acetyl[^{14}C]Phe–[^{3}H]Phe-tRNA–80S ribosome complex was determined by measurement of the radioactivity of the dipeptide *N*-acetyl[^{14}C]Phe–[^{3}H]Phe released from the complex by alkaline hydrolysis. After addition of 1.0 ml of 0.12 M KOH and further incubation for 30 min at 37°, the samples were acidified with 0.1 ml of concentrated (12 M) HCl. Ethyl acetate, 1.5 ml, was then added and the contents of the tube were thoroughly mixed with a Vortex omnimixer. After separation of the phases by low-speed centrifugation, the ethyl acetate layer was collected by suction and evaporated to dryness. The residue was redissolved in 0.2 ml of ethyl acetate, applied to a strip (2 × 30 cm) of Whatman 3 MM paper, and subjected to electrophoresis in 0.05 M pyridine acetate (pH 3.5) at 45 V/cm for 2 hr. *N*-acetyl[^{14}C]phenylalanine was used as marker. After drying, the strips were scanned for radioactivity in a Nuclear Chicago Actigraph III (model 102). The mobility towards the anode of *N*-acetylPhe was 13 cm, that of *N*-acetylPhe-Phe was 9 cm. The strips were cut into 1 × 2-cm pieces, and the radioactivity of each piece was determined in Omnifluor in a Packard Tri-Carb liquid scintillation spectrophotometer. The ^{14}C and ^{3}H radioactivity of the dipeptide region was then calculated with appropriate corrections for counting efficiency and quenching. The counting efficiency was ^{14}C, 75%, ^{3}H, 30% when radioactivity retained on Millipore filters (ribosomal binding) was counted and ^{14}C, 63%, ^{3}H, 3.5% when electrophoresis paper strips (dipeptide formation) were counted. For determination of radioactivity insoluble in hot trichloroacetic acid, the samples were treated with 3 ml of 5% acid, heated to 90° for 15 min, cooled, and filtered through Millipore filters. The filters were thoroughly washed with 5% trichloroacetic acid, dried, and counted in Omnifluor as above.

Miscellaneous. Phe-tRNA and fMet-tRNA labeled with ^{14}C in the phenylalanine and methionine residues, respectively, and Phe-tRNA labeled with ^{3}H in the phenylalanine residue, were prepared by aminoacylation of crude *E. coli* W tRNA as described (1). [^{14}C]Met-tRNA$_f$ was prepared with highly purified *E. coli* tRNA$_f^{Met}$, with an accepting capacity of 1413 pmol/A_{260} unit (1). Although the reacting species of tRNA^{Met} in the experiments reported here is tRNA$_f^{Met}$, we shall use fMet- or Met-tRNA to designate the species prepared from crude tRNA and fMet- or Met-tRNA$_f$ to designate the one prepared from highly purified tRNA. *N*-acetyl[^{14}C]-Phe-tRNA was prepared by the procedure of Haenni and Chapeville (10). [^{14}C]Phe-tRNA was lyophilized to dryness, redissolved in glass-distilled water, and acetylated. The reaction mixture (1 ml) was applied to a column (1 × 22 cm) of Sephadex G-25 (medium grade) equilibrated with 0.01 M acetate buffer (pH 5.5) and eluted with the same buffer. Radioactive fractions that eluted at the void volume were pooled. *N*-acetyl[^{14}C]phenylalanine, used as an electrophoresis marker, was prepared by acetylation of [^{14}C]phenylalanine with acetic anhydride. The ribonucleoside triphosphates ATP, CTP, UTP, GTP, and ITP were obtained from P-L Biochemicals. Other preparations were as in previous work (1).

RESULTS

Lack of GTP requirement

As already reported (1, 2), the reactions catalyzed by the *Artemia* supernatant factor required no addition of GTP. This was quite surprising because the supernatant factor is the eukaryotic counterpart of the prokaryotic initiation factor IF-2, which functions with a stringent and specific requirement for GTP or its analog GMP-PCP. The A_{280}/A_{260} ratios of factor preparations, 1.5–1.7, appeared to exclude the presence of bound nucleotide. Moreover, contamination with GTP was unlikely because fractions having both initiation factor and elongation factor EF-1 activity required the addition of GTP to promote the (EF-1-dependent) poly(U)-directed binding of Phe-tRNA to *Artemia* 80S ribosomes. Since ATP is reportedly required for initiation in a wheat-embryo system (11), the initiation reaction catalyzed by the supernatant factor could be dependent on a nucleoside triphosphate other than GTP. However, one must conclude from the data of Table 2 that there is no nucleoside triphosphate requirement. Other investigators noted the lack of GTP dependence for ribosomal binding of aminacyl-tRNA promoted by rat-liver supernatant factor (3, 5, 6) and the binding of fMet-tRNA promoted by rabbit reticulocyte initiation factor IF-M$_1$ (ref. 7, see also ref. 2).

Reaction with acylated and nonacylated aminoacyl-tRNA

In the first paper of this series (1) it was shown that, besides the AUG-dependent binding of *E. coli* fMet-tRNA, the *Artemia* supernatant factor promoted the poly(U)-dependent binding of *E. coli* Phe-tRNA to 40S subunits. However, whereas the bulk of the bound fMet-tRNA was converted to fMet-puromycin, little Phe-puromycin was formed from the bound Phe-tRNA upon addition of 60S subunits. In order to supplement this information, we have now made a comparative study of the effect of the *Artemia* supernatant factor on the ribosomal binding and puromycin reactivity of *E. coli* fMet-tRNA, Met-tRNA$_f$, *N*-acetylPhe-tRNA, and Phe-tRNA. Study of *N*-acetylPhe-tRNA was important because, at low Mg^{2+} concentrations, it functions as an initiator with

E. coli ribosomes and has the same initiation factor requirements as fMet-tRNA$_f$ (8). The results of these experiments, given in Table 3, show that the nonacylated compounds (Met-tRNA$_f$ and Phe-tRNA) are bound to a smaller extent than the *N*-acylated ones (fMet-tRNA and *N*-acetylPhe-tRNA), and the bound aminoacyl-tRNAs are less reactive with puromycin than the *N*-acylaminoacyl derivatives. It may also be seen in Table 3 that addition of GTP does not improve the reactivity of Met-tRNA$_f$ and Phe-tRNA. Similar results, to be reported elsewhere, have been obtained with *A. salina* Met-tRNA$_f$, fMet-tRNA$_f$, Phe-tRNA, and *N*-AcetylPhe-tRNA.

The low puromycin reactivity of Met-tRNA$_f$ and Phe-tRNA cannot be explained by the assumption that the supernatant factor promotes the binding of the nonacylated derivatives to a site of a 40S subunit from which it can enter the aminoacyl, rather than the peptidyl, site of a 60S subunit upon formation of an 80S couple. Were this the case, one would expect to increase the puromycin reactivity to the level seen with the *N*-acylaminoacyl-tRNAs when GTP and transolcase (elongation factor EF-2) are present along with the supernatant factor. However, addition of GTP and EF-2 did not improve the puromycin reactivity of the bound aminoacyl-tRNA (Table 3, experiments 4 and 6). Thus, it must be assumed that, just as it does with fMet-tRNA and *N*-acetylPhe-tRNA, the supernatant initiation factor promotes the binding of Met-tRNA and Phe-tRNA to an initiation site on a 40S subunit. Since the nonacylated derivatives are bound to a lesser extent than the *N*-acylated ones, it may be that the affinity of the nonacylated derivatives for this site, or for the initiation factor, or both, is lower than that of the acylated. The low puromycin reactivity of the nonacylated aminoacyl-tRNAs may be due to structural requirements of peptidyl transferase. This will be discussed below.

In agreement with the fact that *E. coli* tRNA^{Phe}, normally a carrier of internal phenylalanyl residues, can function as an initiator tRNA in model systems, we find that with *A. salina* ribosomes and factors, Phe-tRNA can be recognized both by the supernatant initiation factor or by the elongation factor EF-1. This contrasts with the behavior of tRNA$_f^{Met}$, which is recognized exclusively by the supernatant factor. As seen in Table 4, there is little or no AUG-dependent binding of fMet-tRNA$_f$ to 80S (40S + 60S) ribosomes, and no Met-puromycin synthesis whether GTP, EF-1, and EF-2 are present or absent. On the other hand, there is poly(U)-dependent binding of Phe-tRNA to ribosomes if GTP and EF-1 are present, and some of the bound aminoacyl-tRNA is converted to Phe-puromycin upon the further addition of EF-2. Here, Phe-tRNA is bound to the aminoacyl site in a GTP- and EF-1-dependent reaction, and must undergo GTP- and EF-2-dependent translocation to the peptidyl site in order to become puromycin-reactive.

TABLE 2. *Lack of nucleoside triphosphate requirement for AUG-dependent binding of fMet-tRNA to 40S ribosomal subunits and fMet-puromycin synthesis*

Additions	f[^{14}C]Met-tRNA bound (pmol)	f[^{14}C]Met-puromycin synthesis (pmol)	(% of bound)
None	4.0	3.3	82
GTP	3.6	3.1	86
ITP	3.5	2.7	77
ATP	3.8	3.0	79
UTP	3.5	3.0	86
CTP	3.5	3.0	86

Duplicate samples were first incubated for 30 min at 0°, under the conditions of the standard assay, without or with nucleoside triphosphate addition (each 0.3 mM) as indicated. Then they were incubated for a further 15 min at the same temperature with the addition of 0.65 A_{260} unit of 60S ribosomal subunits. Just before the second incubation, one of the duplicate samples received 75 μg of puromycin, whereas the other received an equal volume of water. The former served to measure synthesis of of fMet-puromycin, the latter to determine the ribosomal binding of fMet-tRNA. A blank value of 0.5 pmol (both for ribosomal binding and fMet-puromycin synthesis) in the absence of supernatant factor was subtracted throughout.

TABLE 3. *Ribosomal binding and puromycin reactivity of various aminoacyl-tRNAs*

Aminoacyl-tRNA	Factor	GTP	Amino-acyl-tRNA bound (pmol)	Aminoacyl-puromycin synthesis (pmol)	(% of bound)
fMet-tRNA	−	−	0.4	0.3	—
	+	−	4.6	3.2	71
	+	+	4.5	3.2	72
N-acetylPhe-tRNA	−	−	0.6	0.5	—
	+	−	7.3	5.7	78
	+	+	6.6	5.7	86
Met-tRNA$_f$	−	−	0.4	0.1	—
	+	−	2.1	0.5	24
	+	+	2.2	0.5	23
Met-tRNA$_f$*	+	−	3.6	0.3	8
	+	+	3.6	0.4	11
Phe-tRNA	−	−	0.5	0.2	—
	+	−	3.6	0.9	25
	+	+	3.6	0.8	22
Phe-tRNA†	−	+	0.2	0.4	—
	+	+	3.0	1.1	37
	+	+	3.0	0.8	27

Duplicate samples were incubated, under the conditions of the corresponding standard assays, with *N*-acylaminoacyl-tRNAs or aminoacyl-tRNAs, without or with supernatant factor, 0.26 μg, and without or with GTP, 0.3 mM, as indicated, and assayed for binding and puromycin reactivity of the tRNA derivative as described for Table 2.

* The sample with GTP had also 15 μg of EF-2.

† The last sample (with both factor and GTP) also had 15 μg of EF-2.

Stepwise elongation of polypeptide chain

The formation of a true initiation complex by *N*-acetylPhe-tRNA with *A. salina* ribosomes was made use of in a step-by-step study of the requirements for chain elongation with eukaryotic ribosomes and factors. The reaction used was the poly(U)-directed synthesis of an oligopeptide, *N*-acetylPhe-(Phe)$_n$. Similar studies have been done with bacterial systems (12–15). Table 5 shows the simple, but stringent, requirements

TABLE 4. *Effect of GTP and chain-elongation factors on 80S ribosomal binding and puromycin reactivity of Phe-tRNA and Met-tRNA$_f$*

Aminoacyl-tRNA	GTP	EF-1	EF-2	Aminoacyl-tRNA bound (pmol)	Aminoacyl-puromycin synthesis (pmol)
Phe-tRNA	−	+	+	0.4	0
	+	−	+	0	0
	+	+	−	7.3	0
	+	+	+	7.0	2.5
Met-tRNA$_f$	−	+	+	0.2	0
	+	−	+	0.2	0
	+	+	−	0.2	0
	+	+	+	0.1	0

Incubations and assays were as in Table 3, except that both 40S and 60S ribosomal subunits (0.27 and 1.1 A_{260} units, respectively) were present and no supernatant initiation factor was added. Where indicated, GTP (0.3 mM), EF-1 (15 μg), and EF-2 (15 μg) were added. The incubation temperature was 25° in the experiment with Phe-tRNA [poly(U) as messenger], and 0° in the experiment with Met-tRNA$_f$ (AUG as messenger). A 0.3 pmol blank (no GTP and no elongation factor additions) was subtracted throughout the Phe-tRNA experiment.

of the initiation step. In this model system, initiation requires a single initiation factor—the supernatant factor—, is GTP-independent, and occurs exclusively on free 40S subunits. 80S ribosomes (Table 5) and, as reported earlier (1), 60S subunits are completely inactive. As seen in Table 6, after the addition of 60S subunits, the second aminoacyl-tRNA (Phe-tRNA) is bound, with a strict requirement for elongation factor EF-1 and GTP. This is followed by peptide-bond synthesis, with formation of an *N*-acetylPhe-Phe-tRNA–80S

TABLE 5. *Sequential elongation of polypeptide chain. Step 1: formation of 40S initiation complex*

Basic system	Further additions	Ribosomal binding of *N*-acetyl-[^{14}C]Phe-tRNA (pmol)
A. 40S + poly(U) + *N*-acetyl[^{14}C]Phe-tRNA	None	0.4
	Factor	6.5 (6.1)
	Factor + GTP	6.1 (5.7)
	EF-1 + GTP	0.7 (0.3)
B. 80S + poly(U) + *N*-acetyl[^{14}C]Phe-tRNA	None	0.3
	Factor	0.1 (0.0)

A. Conditions were those of the standard assay with *N*-acetylPhe-tRNA.

B. Same as *A*, except that the 40S ribosomal subunits were replaced by a mixture of 40S and 60S subunits (0.17 and 0.37 A_{260} units, respectively). Additions: Supernatant factor, 0.26 μg; GTP, 0.3 mM; EF-1, 15 μg. Values in parentheses are net values after subtraction of blanks without factor.

TABLE 6. *Sequential elongation of polypeptide chain. Step. 2: formation of 80S initiation complex, binding of second aminoacyl-tRNA, and formation of first peptide bond*

System	Ribosomal binding of *N*-acetyl[^{14}C]-Phe-tRNA (pmol)	Ribosomal binding of [^{3}H]Phe-tRNA (pmol)
No factor, no EF-1	0.7	0.2
No GTP, no EF-1	6.1 (5.3)	0.4 (0.2)
No GTP	6.3 (5.6)	0.9 (0.7)
No EF-1	6.3 (5.6)	0.4 (0.2)
Complete	6.4 (5.7)	5.6 (5.4)

System	Amino acids in dipeptide *N*-acetylPhe-Phe: *N*-acetyl[^{14}C] Phe (pmol)	Amino acids in dipeptide *N*-acetylPhe-Phe: [^{3}H]Phe (pmol)
No 60S subunit	0	0
Complete	4.2	3.9

N-acetyl[^{14}C]Phe-tRNA was bound to 40S ribosomal subunits as in Table 5 (*A*). The tubes were then placed in an ice–water bath and the following components were added to each sample to give the indicated final concentrations or amounts: 60S ribosomal subunits, 1.1 A_{260} units; [^{3}H]Phe-tRNA (5700 cpm/pmol), 24 pmol; and, when present, GTP, 0.6 mM, and EF-1, 15 μg. After incubation for an additional 30 min at 25°, the samples were assayed for aminoacyl-tRNA binding (upper part of Table) and dipeptide formation (lower part of Table). The dipeptide values were corrected for 64% recovery of *N*-acetyl[^{14}C]Phe-tRNA in the ethyl acetate phase. Values in parentheses are net values after subtraction of blanks without factor.

ribosome complex. This was characterized as the dipeptide *N*-acetylPhe-Phe (Table 6) after its release by brief incubation at pH 10.0. Elongation factor EF-2 is not required in this step. However, further elongation of the polypeptide chain depends on the occurrence of translocation and, as shown in Table 7, is obtained only after addition of EF-2.

DISCUSSION

From the results presented in this paper, we conclude: (*a*) a functional, AUG-dependent initiation complex is formed by interaction of *E. coli* fMet-tRNA$_f$ with eukaryotic 40S ribosomal subunits. The criterion for functionality is extensive conversion of the bound *N*-acyl-aminoacyl-tRNA to the

TABLE 7. *Sequential elongation of polypeptide chain: Translocation and further elongation*

	Acid-insoluble material: *N*-acetyl[^{14}C]-Phe (pmol)	Acid-insoluble material: [^{3}H]Phe (pmol)
Without EF-2	0.03	0.1
With EF-2	1.6	7.6

15 μg of EF-2 was added to one of two identical samples, previously incubated as in the complete system of Table 6 so as to form the *N*-acetyl[^{14}C]Phe-[^{3}H]Phe-tRNA–poly(U)–80S complex and, after further incubation (10 min at 25°), the incorporation of ^{14}C and ^{3}H radioactivity into material insoluble in hot trichloroacetic acid was determined.

dipeptide analog fMet-puromycin upon addition of puromycin and 60S subunits. (*b*) An analogous poly(U)-dependent and puromycin-reactive complex is formed with *E. coli* *N*-acetyl-Phe-tRNA. (*c*) The above reactions are catalyzed by a single initiation factor and, unlike their prokaryotic counterparts, have no requirement for GTP or other nucleoside triphosphates. (*d*) Substitution of Met-tRNA$_f$ for fMet-tRNA$_f$ or of Phe-tRNA for N-acetylPhe-tRNA results in reduced complex formation and decreased puromycin reactivity.

The low puromycin reactivity of the nonacylated aminoacyl-tRNAs has been noted by several investigators, not only with *E. coli* (16, 17, 19) but also with eukaryotic ribosomes (18–20). It may reflect the requirement by peptidyl transferase that a substrate at the peptidyl, i.e., the donor site, have an amide rather than a free α-amino group (16, 17). This requirement is met by prokaryotes, where the initiator is fMet-tRNA$_f$ rather than Met-tRNA$_f$, but not by eukaryotes, where the situation is apparently reversed (21). The mechanism of initiation in eukaryotes, with Met-tRNA$_f$ as the initiator, is poorly understood. In contrast to the simple requirements obtaining in our studies with fMet-tRNA$_f$ (or *N*-acetylPhe-tRNA) as initiator, no fewer than three factors (IF-M$_1$, IF-M$_{2A}$, IF-M$_{2B}$) are required, besides GTP, for the AUG-dependent binding of Met-tRNA$_f$ to reticulocyte ribosomes (7, 22, 23).

Although both the ribosomal subunits and the initation factor used by us stemmed from encysted embryos of the brine shrimp *Artemia salina*, similar initiation factors are found in mammalian cells (2). In fact, the *Artemia* and rat-liver ribosomes and supernatant initiation factors are fully interchangeable, and preparations of the reticulocyte factor IF-M$_1$ readily promote the formation of a functional initiation complex with fMet-tRNA or *N*-acetylPhe-tRNA and *Artemia* ribosomes (2). The use of *Artemia* embryos is advantageous for several reasons: (*a*) ribosomal subunits are easily prepared and are very active (*b*) the formation of an initiation complex with *Artemia* ribosomes has an absolute requirement for the free, small ribosomal subunit (Table 5) not easily demonstrable with other eukaryotic or *E. coli* ribosomes, and (*c*) brine shrimp eggs are readily available and very suitable for large-scale preparation work.

Artemia ribosomes and factors as also well suited for the study reported in this paper of the stepwise elongation of the polypeptide chain with the poly(U)-dependent synthesis of *N*-acetyl-Phe-(Phe)$_n$ as a model system. As expected, the process is basically the same as in *E. coli* (12, 14, 15), except for the lack of GTP requirement in the initiation step.

It may be noted in closing that whereas some limited interchangeability of prokaryotic and eukaryotic chain-elongation factors has been noted (24, 25, and H. C. Klein and S. Ochoa, in preparation), the *Artemia* supernatant initiation factor and *E. coli* initiation factor IF-2 are not interchangeable (2). On the whole, there have not been very pronounced changes in the evolution of systems of protein biosynthesis from the prokaryotic to the eukaryotic type, except perhaps for the initiation step. A better understanding of the mechanism of of eukaryotic initiation will, therefore, be of considerable biological importance.

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